Phage Australia

THERAPEUTIC USE OF BACTERIOPHAGE PREPARATION EC184B

Phage Australia | Jon Iredell

Phage Australia

Phage Safety Data Sheet v2.0

Issued	Westmead	**time**	23092022_1430
Common name	EC134B		caudovirales/ demerecviridae
PA ID	ECP00134B		
Provenance	Westmead (Iredell lab) Jan 2019		
Genomic. acc. no.	TBA		

Lysogeny markers identified[1]	None
Antibiotic resistance genes identified[2]	None
Virulence related genes identified[3]	None

Comment

EC134B is a dsDNA T5 virus of 108 Kb, with no evident virulence- or antibiotic resistance-associated genes of concern detected, using standard approaches at the time of reporting*. Its closest match in NCBI *nr* is Salmonella phage L6jm (89% coverage, 98% max identity) at the time of reporting. Extremely high coverage (>900X) was obtained to ensure absence of background contamination.

Reporting bioinformatician **Nouri BEN ZAKOUR**

Supervising pathologist  **Jon IREDELL 24/09/22**

**Genome sequencing*: Whole genome sequencing (WGS) of pure phage preparation was performed at the Centre for Infectious Disease and Microbiology, Westmead Institute for Medical research. Genomic DNA was extracted using QIAGEN DNeasy Blood and Tissue Mini Kit (QIAGEN). Sequencing libraries were prepared using Illumina DNA Library Prep Kit and sequenced on a iSeq 100.
Primary genome analysis: Sequenced raw reads were subjected to an in-house quality control procedure prior to further analysis and analyzed using the in-house bz_phage_pipeline (v0.1.0). Sequencing quality assessment and filtering was performed using FastP (v0.20.1). Assembly was performed using Unicycler v0.5.0 (conservative and normal modes) and inspected using Bandage (v0.8.1). Taxonomy and sequence contamination profiling were performed using Kraken 2 (v2.1.2 – db minikraken2_v2_8GB_201904_UPDATE) and Blobtools (v1.1.1 – db ref_viruses_rep_genomes 2022-Sep-23 update). Closest match was identified using BLASTn (v2.13.0) against nr. Identification of phage-related contigs, functional annotation, lifestyle predictions, phage contigs assembly quality, and circularity were performed using the VIBRANT pipeline (v1.2.0).
Resistome analysis: Sequence data were screened using Abricate (v1.0.1) against the NCBI Bacterial Antimicrobial Resistance Reference Gene Database (2022-Sep-23 update) to identify specific resistance gene variants. Results from the VIBRANT pipeline were also used to confirm absence of resistance related genes using HMMER3 against KEGG, PFAM and pVOG.
Virulence factors analysis: Sequence data were screened using Abricate (v1.0.1) against the VFDB (2022-Sep-23 update). Results from the VIBRANT pipeline were also used to confirm absence of virulence and toxin related genes using HMMER3 against KEGG, PFAM and pVOG.

Enquiries to **Jon Iredell**

02 8890 6102/6255

Phage Australia Bacterial Safety Data Sheet v2.0

Issued	Westmead	**time**	23092022_1430
Common name	BW25113	*Escherichia coli*	
PA ID	BW251131-1	ST 10	
Genomic. acc. no.		CP009273	
prophages identified[1]		CP4-6, DLP12, e14, rac, Qin, CP4-44, CPS-53, CPZ-55, CP4-57	
major plasmids identified[2]:		none	
Antibiotic resistance genes identified[3]		blaEC-19	
Virulence related genes identified[4]		none	

Comment

The clinical risk associated with co-purified toxins and phages arising from this production strain is low.

Escherichia coli strain BW25113 is a K12 laboratory strain extensively studied and used for phage propagation, systematic phenotypic surveys and synthetic biology projects. Nine prophages are detected (mostly remnant, 10-40 Kb). Genome sequence analysis of this strain using standard approaches at the time of reporting* revealed an absence of problematic antimicrobial resistance genes, characterised toxins, plasmids, and functional CRISPR machinery. BlaEC-19 is a chromosomal class C cephalosporinase-encoding gene ("*E. coli* Amp C") commonly found in *E. coli*.

Reporting bioinformatician **Nouri BEN ZAKOUR**

Supervising pathologist  **Jon IREDELL 24/09/22**

**Genome sequencing*: Whole genome sequencing (WGS) of pure cultures was performed at the Centre for Infectious Disease and Microbiology, Westmead Institute for Medical research Genomic DNA was extracted using QIAGEN DNeasy Blood and Tissue Mini Kit (QIAGEN). Sequencing libraries were prepared using Illumina DNA Library Prep Kit and sequenced on an iSeq 100.

Primary genome analysis: Sequenced raw reads were subjected to an in-house version of the Nullarbor pipeline (v1.2; https://github.com/tseemann/nullarbor). Assemblies were performed using Unicycler v0.5.0 and inspected using Bandage (v0.8.1). Taxonomy and sequence contamination profiling were performed using Kraken 2 (v2.1.2 – db minikraken2_v2_8GB_201904_UPDATE) and Blobtools (v1.1.1 – db ref_viruses_rep_genomes 2022-Sep-23 update). The WGS-based multilocus sequence type (MLST) was inferred from sequencing data using MLST 2.8 from the pipeline.

Mobile genetic elements analysis: Identification of phage-related contigs, functional annotation, lifestyle predictions, phage contigs assembly quality were performed using the VIBRANT pipeline (v1.2.0). Additional intact and remnant prophages were identified using PHASTER (https://phaster.ca/), then manually inspected.

Resistome analysis: Sequence data were screened using Abricate (v1.0.1) against the NCBI Bacterial Antimicrobial Resistance Reference Gene Database (2022-Sep-23 update) to identify specific resistance gene variants. Characteristics and mobile genetic elements and plasmid markers were inferred from sequencing data using the Plasmid Finder and plasmidMLST services (https://cge.cbs.dtu.dk/services/).

Virulence factors analysis: Sequence data were screened using Abricate (v1.0.1) against the VFDB (2022-Sep-23 update). Results from the VIBRANT pipeline were also used to confirm absence of virulence and toxin related genes using HMMER3 against KEGG, PFAM and pVOG.

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Phage Australia Batch Cert. Data Sheet v2.0

Issued	WIMR	**time**	27092022_2210
Phage	Ec134B		Caudovirales/ Demerecviridae
Production strain	BW25113		*Escherichia coli*

Batch ID	**Ec134b_BW25113_270922**
Production date	**27092022**
Nominated expiry date	**25102022**
Medium	saline
Storage	4C, glass vials
Batch volume	34 x 2.0-mL vials
PFU/mL	1 x 10^9
EU/mL	6.18
Sterility	No growth at 5 days
pH	6.6
Genome sequencing*	99.7% sequence purity

Comment

Phage Ec134b was produced and purified according to Phage on Tap methodology (Bonilla et al. 2016)**. Briefly, phage was co-cultured with host strain BW25113 in 150 mL LB + 1mM $CaCl_2$ + 1mM $MgCl_2$ at 37 ºC 18 h to a final concentration of 1.1x10^{11} PFU/mL. Clarified lysate was filtered through 0.22 um to sterilize, concentrated by 50 kDa Amicon spin column, and washed with saline to remove media components. Endotoxin was depleted using octanol extraction. Octanol was removed by dialysis against 25% EtOH (2L x 3 h x 4) and then 0.15 M NaCl (2L x 3 h x 4). Endotoxin reading of concentrated lysate (measured by Charles River Limulus Amoebocyte Lysate assay using Endosafe LAL cartridge) showed 618 EU/mL. Batch was diluted 1/100 in sterile saline, aliquoted into 2 mL vials, and stored at 4 ºC. Phage titre of diluted lysate (as determined by plaque assay on BW25113) showed 1.0 x 10^9 PFU/mL. Diluted batch was used to inoculate blood culture bottles for sterility testing (1-mL inoculum; aerobic and anaerobic) and to perform pH testing. Sterility testing was performed by Institute Of Clinical Pathology And Medical Research (ICPMR), Westmead via Culture Body Fluid BC Bottle.

Batch satisfies endotoxin thresholds for safe use at up to 10^9 PFU/kg/hr intravenously based on <5 EU/kg/h acceptable limit.

Reporting scientists **Jessica SACHER**

Supervising pathologist  **Jon IREDELL 04/10/22**

**Genome sequencing*: Whole genome sequencing (WGS) of pure phage preparation was performed at the Centre for Infectious Disease and Microbiology, Westmead Institute for Medical research. Genomic DNA was extracted using QIAGEN DNeasy Blood and Tissue Mini Kit (QIAGEN). Sequencing libraries were prepared using Illumina DNA Library Prep Kit and sequenced on a iSeq 100.

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Resistome analysis: Sequence data were screened using Abricate (v1.0.1) against the NCBI Bacterial Antimicrobial Resistance Reference Gene Database (2022-Sep-23 update) to identify specific resistance gene variants. Results from the VIBRANT pipeline were also used to confirm absence of resistance related genes using HMMER3 against KEGG, PFAM and pVOG.

Virulence factors analysis: Sequence data were screened using Abricate (v1.0.1) against the VFDB (2022-Sep-23 update). Results from the VIBRANT pipeline were also used to confirm absence of virulence and toxin related genes using HMMER3 against KEGG, PFAM and pVOG.

**Bonilla N, Rojas MI, Netto Flores Cruz G, Hung S, Rohwer F, Barr JJ. 2016. Phage on tap–a quick and efficient protocol for the preparation of bacteriophage laboratory stocks. PeerJ 4:e2261 https://doi.org/10.7717/peerj.2261

Phage Australia Phage-bacteria Matching Data Sheet v2.0

Issued	Westmead	**time**	26092022_12:30
Referring doctor	Jon Iredell		Westmead Hospital, NSW
Contact	02 8890 6012		
Received	29032021_1140		
Case ID	**WIMR_C6**		
Isolate ID	ECOL_C6_1 & ECOL_C6_2		

Clinical notes
Chronically infected thoracotomy dominated by *E. coli* pathogen, previous combined antibiotic and surgical approaches have failed.

In vitro phage susceptibility

Efficiency of plating (EOP)

Efficiency of plating (EOP) is used to determine the efficiency of a phage on a test isolate (e.g. patient isolate) compared to the phage reference strain (host strain). The EOP is determined by serially diluting a phage of interest of a known concentration and spotting the dilutions on a bacterial lawn of a reference strain (phage host strain) and on a test isolate (e.g. patient isolate). Following incubation, the titre of the phage on the respective bacterial isolates is calculated by counting the number of phage plaques, multiplied by the dilution factor and divided by the volume of phage plated. The EOP value is calculated by dividing the titre of the phage on the test isolate by the titre on the reference strain divided, this value is represented as a percentage of efficiency.

Titre of EC134b on BW25113 (EOP %): 1.1×10^{11} PFU/mL (100%)

Titre of EC134b on Case 6 isolate 1 (EOP %): 1.03×10^{11} PFU/mL (94%)

Titre of EC134b on Case 6 isolate 2 (EOP %): 0 PFU/mL (0%)

Kinetic assay

A kinetic assay is an assay used to observe the phages' ability to inhibit the bacterial growth within a liquid medium. For this assay, the patient bacterial isolate is added to wells of a 96-well microplate, followed by the addition of either control substances (broth) or phage at different concentrations, and the absorbance (OD 600nm) is read every 10 mins over an 18-hour period, by a microplate reader. Following the 18-hour assay, analysis is performed to determine whether the phage is able to inhibit the patient isolate and the optimal phage concentration to do so.

Enquiries to **Jon Iredell**

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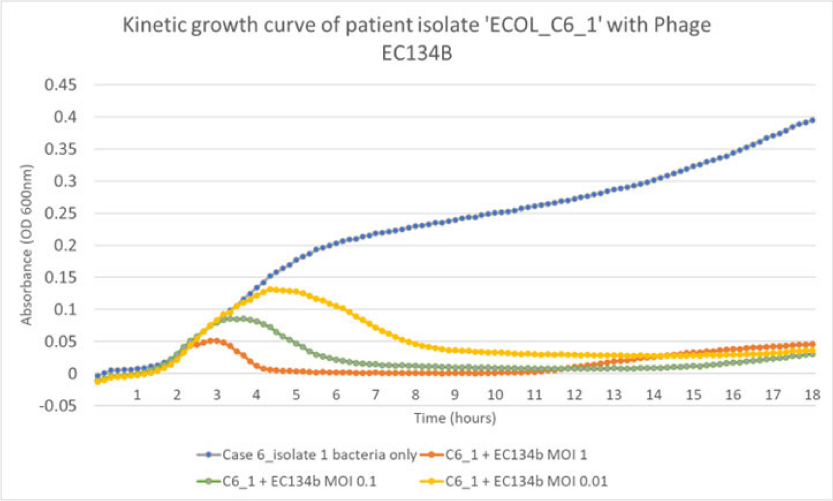
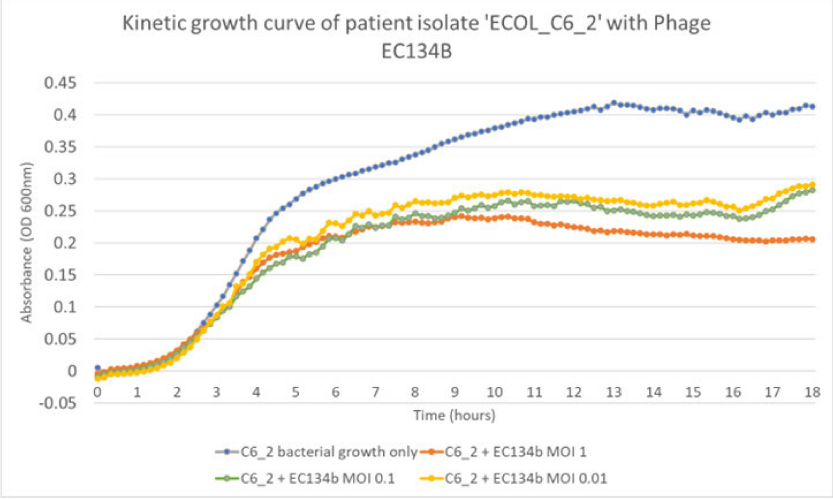
Phage Australia Phage-bacteria Matching Data Sheet v2.0





Comment

Escherichia coli ECOL_C6_1 is susceptible to EC134b with a 94% efficiency in the EOP assay and inhibits the growth of this isolate for 18 hours in the kinetic assay (Figure 1).
Escherichia coli ECOL_C6_2 is not susceptible to EC134b as there is a 0% efficiency in the EOP assay, however, there is a moderate inhibitory effect of this phage-isolate combination in the kinetic assay (Figure 1).

Enquiries to **Jon Iredell**

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Recommend.
EC134b at MOI 1 (~1 x 10^9 PFU/mL) adjunct to antibiotics recommended by treating physician.

Reporting scientists **Stephanie LYNCH & Jessica SACHER**

Supervising pathologist  **Jon IREDELL xx/xx/22**

Enquiries to **Jon Iredell**

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